Clean Copy of Page 9, Paragraph on Lines 27-29

a2

Figures 10A-B show pictures of tissue samples which have been H&E stained using either the routine fixation and processing method (no ultrasound) (Figure 10A) or the new technique (with ultrasound) (Figure 10B).

Clean Copy of Page 10, Paragraph on Lines 1-3

a3

Figures 11A-B show pictures of tissue samples which have undergone CD5 staining using the routine fixation and processing method (no ultrasound) (Figure 11A) or the new technique (with ultrasound) (Figure 11B).

Clean Copy of Page 10, Paragraph on Lines 4-6

a4

Figures 12A-B show tissue sections which have undergone in situ hybridization with poly-A mRNA using either the routine fixation and processing method (no ultrasound) (Figure 12A) or the new technique (with ultrasound) (Figure 12B).

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a5

The sections from ultrasound treated tissue were excellent in histologic appearance as compared with their routine fixation and processing counterparts. The color balance in the H&E ultrasound section consistently demonstrated slightly more eosinophilia on the cytoplasm and more intense nucleus staining than the routine method (Figures 10A-B). All ultrasound irradiated tissue blocks sectioned as well as control tissue blocks and no difference was detected in the sectioning and staining process. No evidence of cavitation tissue injury was noted in the ultrasound treated specimens under the conditions employed in this study. Ultrasound treated tissue sections following protease or MW antigen retrieval pretreatment showed no disintegration or deterioration. This indicates that tissue is fixed by formalin rather than by alcohol (dehydration only 10-15 minutes) according to Azumi's explanation (Azumi et al., 1990).

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a6

The distribution of IHC for CD45, CD20, CD3, CD5, Bcl-2, cytokeratin, kappa and lambda from routine or ultrasound treated tissue sections is similar in this study. Several of the factors involved in the process of fixation were found to affect immunoreactivity of the antibodies used in this study. These include the duration and the speed of fixation and processing, and the duration and the concentration of primary and secondary (2°) antibody incubation. A short incubation with primary/2° antibodies/ABC (10 minutes/5 minutes/5 minutes) gave poor staining results compared to the overnight incubation. However, this method gave the best measurement to evaluate the condition of antigen preservation. The tissue from ultrasound irradiated fixation and processing significantly improved the immunoreactivity of the majority antibodies (CD3) in this study, and also dramatically reduced the incubation time. The requirement of concentration of primary antibodies (cytokeratin) for ultrasound treated tissue also was reduced more 20-fold compared to the routine treated tissue. Ultrasound high-speed fixed and processed tissues demonstrated the optimal fixation condition that was stained by CD5 even without MW antigen retrieval pretreatment (Figures 11A-B).

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a7

ISH is an excellent method for visualization and accurate detection of a specific gene (e.g., oncogene, tumor suppressor gene or viral gene) in individual, morphologically defined normal and neoplastic cells in both fresh and archival tumor specimens with light microscopy. mRNA ISH is one of the best methods to evaluate the condition of tissue mRNA preservation (Weiss and Chen, 1991; Harper et al., 1992). Since the poly d(T) probe presumably hybridizes to polyadenylated sequences of RNA, it would be expected that this probe would hybridize to the majority of mRNA species – only 10-30% of mRNA lack the polyadenylated tail. Ultrasound high-speed fixed and processed tissue dramatically improved the total polyadenylated mRNA preservation more than 20-fold in the periphery and more than 100-fold in the center of tissue as compared with the routine method (Figures 12A-B). As a check on the validity of the poly d(T) used to detect mRNA, we performed parallel studies to detect a specific mRNA, using the probes recognizing kappa immunoglobulin light chain mRNA. The even distribution of kappa mRNA protected was found in the tissue treated by the ultrasound method. However, in the tissue treated with the routine

Cont a7

method, the periphery and center showed uneven distribution, i.e., there was good fixation of the tissue at the periphery and good preservation of mRNA at the periphery due to inactivation of RNAse in that region therefore showing good results, but the more interior regions of the tissue were not well fixed and mRNA was not well preserved and showed poor results.

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a8

41. The method of claim 38 wherein said method is performed on a solid phase, a microarray, a membrane or a DNA chip and wherein said solid phase, microarray, membrane or DNA chip receives ultrasound power of at least 0.01 W/cm^2.

Clean copy of Amended Claim 45

45. The method of claim 38 wherein said method is performed on a solid phase, membrane, microarray or DNA chip and wherein one or more ultrasound transducers are used to produce an ultrasound field that allows at least a portion of said solid phase, membrane, microarray or DNA chip to receive a uniform frequency and intensity of ultrasound.

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54. The method of claim 44 wherein said method is performed on a solid phase, membrane, microarray or DNA chip and wherein said transducers are arranged around said solid phase, membrane, microarray or DNA chip in a two-dimensional arrangement.

a10

Clean copy of Amended Claim 55

55. The method of claim 44 wherein said method is performed on a solid phase, membrane, microarray or DNA chip and wherein said transducers are arranged around said solid phase, membrane, microarray or DNA chip in a three-dimensional arrangement.

a11

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a^{12}

56. The method of claim 38 wherein said method is performed on a solid phase, membrane, microarray or DNA chip and wherein said solid phase, membrane, microarray or DNA chip is rotated.

Clean copy of Amended Claim 57

a^{13}

57. The method of claim 38 wherein said method is performed on a solid phase, membrane, microarray or DNA chip and wherein said transducer revolves around said solid phase, membrane, microarray or DNA chip.

Clean copy of Amended Claim 69

a^{14}

69. The method of claim 38 wherein said method is performed on a solid phase, membrane, microarray or DNA chip wherein said solid phase, membrane, microarray or DNA chip receives ultrasound of a power in the range of 0.01-100 W/cm^2.